

REMARKS

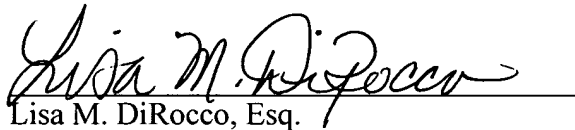
Pages 48, 54, 56, 59 and 60 of the specification have been amended to insert sequence identifiers for the nucleotide sequences recited on those pages, as required in item 1), second paragraph, on page 1 of the Office Communication. Item 1), second paragraph, on page 1 of the Office Communication also indicates that the sequences on page 51 of the specification require sequence identifiers. However, according to 37 CFR § 1.821(a), "Nucleotide and/or amino acid sequences as used in §§ 1.821 through 1.825 is interpreted to mean an unbranched sequence ... of *ten or more* nucleotides." The two sequences recited on page 51 at lines 24 and 29, respectively, have only nine (9) and five (5) nucleotides, respectively. Therefore, because each of these sequences has fewer than ten (10), neither requires a sequence identifier. In addition, the specification has been amended by a direction to enter the paper copy of the substitute Sequence Listing into the specification, as required by the Notice to Comply.

Support for the amendments is found in the specification and in the claims as originally filed. *No new matter has been added to the application.* Attached hereto as Appendix A are amendments to the specification with markings to show the changes made.

CONCLUSION

In view of the foregoing, and allowance of the instant application with all pending claims is respectfully solicited. If a telephone conversation with Applicants' attorney would help expedite the prosecution of the above-identified application, the Examiner is urged to call Applicants' attorney at (617) 227-7400.

Respectfully submitted,
LAHIVE & COCKFIELD, LLP


Lisa M. DiRocco, Esq.
Registration No. 51, 619 for
Peter C. Lauro, Esq.
Registration No. 32,360
Attorney for Applicants

28 State Street
Boston, MA 02109
Tel. (617) 227-7400

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APPENDIX A
VERSION WITH MARKINGS TO SHOW CHANGES MADE

Please amend the specification as follows:

At page 48, please delete the paragraph beginning at line 3 and insert therefor the following paragraph:

--Protein microsequence data from the N-terminus (ATVDTWDRLEExSNGTxD) (SEQ ID NO: 38) and an internal peptide (VGGEYYPHQASK) (SEQ ID NO: 42) obtained from the purified RP-factor were used to design two oligonucleotides, denoted A1 [GCSACSGTSGACACSTGGGACCGSCTSGCSGAG] (SEQ ID NO: 37) and A2 [GCYTGRTGIGGRTAICCYTCICC] (SEQ ID NO: 41), respectively. Taq polymerase was employed under standard conditions to amplify a 147 bp PCR product from *M. luteus* DNA with these primers. The PCR product obtained from *M. luteus* DNA with these two primers was labeled with digoxigenin and used as a probe for Southern hybridization experiments. *Sma*I-digested genomic DNA was size-fractionated by agarose gel electrophoresis and circa 1.4 kbp fragments were cloned in pMTL20 and established in *E. coli* strain DH5a. Two recombinant plasmids carrying the desired insert were detected by hybridization, confirmed by PCR using oligonucleotides A1 and A2, and one of them was manually sequenced on both strands using the dideoxy chain termination method.--

At page 54, please delete the paragraph beginning at line 21, and insert therefor the following paragraph:

--Two primers [5'-GTCAGAATTCATATGGCCACCGTGGACACCTGGG-3'] (SEQ ID NO: 46) and [5'-TGACGGATCCTATTAGGCCTGCGGCAGGACGAG-3'] (SEQ ID NO: 47) were employed to amplify (5 cycles of 30s at 94°C, 30s at 60°C, 30s at 72°C, followed by 15 cycles of 30s at 94°C, 60s at 72°C) the RP-factor coding sequence (i.e. lacking the signal sequence) from the cloned 1.4 kbp *Sma*I fragment of genomic DNA. It was first established in *E. coli* DH5a as a 567 bp *Eco*BI-*Bam*HI fragment in pMTL20 and then excised as a 562 bp *Nde*I - *Bam*HI fragment, inserted into pET19b (Novagen) and re-established in *E. coli* DH5a. The sequence of the PCR product and vector-insert junction in this plasmid, denoted pRPF1, was verified. RP-factor was expressed from RPF1 after transforming it into *E. coli* HSM174(DE3). The protein, containing a His₆-tag at the N-terminus, was isolated by sonicating bacteria, previously grown to an OD_{600nm}=0.6 and induced with 0.4 mM IPTG for 4 h, in a modified binding buffer (MBB - 5mM imidazole pH7.9/0.5M NaCl/20mM Tris-HCl/8M urea) containing 5 mM DTT and 2 mM EDTA. After low speed centrifugation, low

MW compounds, including EDTA and DTT, were removed by elution through a Sephadex G10 column pre-equilibrated with MBB. A Ni^{2+} -chelation column (Ni^{2+} -coordinated iminodiacetic acid immobilized on Sepharose 6B), was loaded with the G10 eluate, washed with 20 vol MBB and then successively eluted with four 10 vol aliquots of MBB containing 0.01 M, 0.05 M, 0.2 M and 1 M imidazole, respectively. The column was finally eluted with strip buffer (20 mM Tris-HCl, pH 7.9/100 mM EDTA/0.5 M NaCl). Monoclonal anti-(polyHis) antibodies (Sigma, clone His-1) were employed for immunoblot analysis of fractions subjected to SDS PAGE electrophoresis and electroblotted using standard methods. Fractions were dialysed against buffer 2 and assayed for biological activity as indicated above.--

At page 56, please delete the paragraph beginning at line 24, and insert therefor the following paragraph:

--Two primers [5'-ATCAGAATTCATATGGACGACATCGATTGGGACGC-3'] (SEQ ID NO: 48) and [5'-CGCAGGATCCCCTCAATCGTCCCTGCTCC-3'] (SEQ ID NO: 49) were employed to amplify (5 cycles of 30s at 94°C, 30s at 58°C, 30s at 72°C, followed by 25 cycles of 30s at 94°C, 60s at 72°C) the RP-factor coding sequence (*i.e.*, lacking the signal sequence) from *M. tuberculosis* H37Rv genomic DNA. The PCR product was first established in *E. coli* DH5a as a 336 bp *Eco*El-*Bam*HI fragment in pMTL20 and then excised as a 331 bp 30 *Nde*I - *Bam*HI fragment, inserted into pET19b (Novagen) and re-established in *E. coli* DH5a. The sequence of the PCR product and vector-insert junction in this plasmid, denoted pRPF2, was verified. The *M. tuberculosis* RP-factor was expressed from pRPF2 after transforming it into *E. coli* HSM174(DE3). The protein, containing a His₁₀-tag at the N-terminus, was isolated by sonicating bacteria, previously grown to an OD_{600nm} = 0.9 and induced with 0.4 mM IPTG for 4 h, in binding buffer (BB - 5mM imidazole pH7.9 / 0.5M NaCl / 20 mM Tris-HCl / 8M urea). After low speed centrifugation, a Ni^{2+} -chelation column (Ni^{2+} -coordinated iminodiacetic acid immobilised on Sepharose 6B), was loaded with the supernatant, washed with 20 vol BB, 20 vol BB containing 100 mM imidazole, and then eluted with 10 vol BB containing 0.5 M imidazole. Additional purification was achieved by MonoQ column chromatography (*vide infra*, save that the salt gradient was from 0.1 M to 1M NaCl). Monoclonal anti-(polyHis) antibodies (Sigma, clone His-1) were employed for immunoblot analysis of fractions subjected to SDS PAGE electrophoresis and electroblotted using standard methods. Fractions were dialysed against buffer 2 and assayed for biological activity as indicated above.--

At page 59, please delete the paragraph beginning at line 23 and insert therefor the following paragraph:

-- The entire *yabE* coding region together with flanking sequences was amplified from *B. subtilis* genomic DNA using primers D1 1 [5'-GAAGAGAATTCCTTCCATCACGA-3'] (SEQ ID NO: 50) and D12 [5'-CCAAACGAATTCGGTCAATCAC-3'] (SEQ ID NO: 51) as a 1803 bp product. A 1186 bp *HindIII*-*BctL* fragment encompassing the 3' end of the coding sequence was excised from the PCR product, ligated with *HindIII* + JSamHI-digested pMTL20, and used to transform *E. coli* strain DH5a with selection for ampicillin-resistance. Plasmid pYABE was isolated from one of the transformants. A 763 bp //wdIII-.8a/wHI fragment from entirely within the *yabE* coding sequence was excised from the pYABE, ligated with *HindIII* + .BamHI-digested pMUTIN4, an integrating plasmid that may be employed for generating knockout mutations in *B. subtilis* (Edwards & Errington, 1997, Molecular Microbiology, 24,905-915) and used to transform *E. coli* strain XL 1-Blue with selection for ampicillin-resistance. Plasmid pYAB2, containing an internal segment of the *yabE* coding sequence, was isolated from one of the transformants. A 1207 bp *HindIII*-*EcoRI* fragment encompassing the 3' end of the *yabE* coding sequence was excised from pYABE, ligated with *HindIII* + *EcoRI* digested pMUTIN4 and used to transform *E. coli* strain XL1-Blue with selection for ampicillin-resistance. Plasmid pYAB3, containing the 3' end of the *yabE* coding sequence, was isolated from one of the transformants.--

At page 60, please delete the paragraph beginning at line 9, and insert therefor the following paragraph:

--The entire *yocH* coding region together with flanking sequences was amplified from *B. subtilis* genomic DNA using primers D10 [5'- GCAAGGATCCCAGACTAAAAAACAG-3'] (SEQ ID NO: 52) and D9 [5'- ATCAGGATCCATATTATTAGTTTAAGA-3'] (SEQ ID NO: 53) as a 1145 bp product. A 358 bp *Hpa* fragment from entirely within the *yocH* coding sequence was excised from the PCR product, ligated with *Sma*I-digested pMTL20, and used to transform *E. coli* strain XL1- Blue with selection for ampicillin-resistance. Plasmid pYOC2a, containing an internal segment of the *yocH* coding sequence, was isolated from one of the transformants. The insert in this plasmid was then excised from pYOC2a as a 385 bp *Eco*RI-*Hind*III fragment and inserted into pMUTIN4, to yield pYOC2. A 307 bp *Hind*III-*Bam*HI fragment encompassing the 3' end of the *yocH* coding sequence was excised from the 1145 bp PCR product, ligated with *Hind*III + *Bam*HI digested pMUTIN4, and used to transform *E. coli* strain DH5a with selection for ampicillin-resistance. Plasmid pYOC3, containing a DNA segment encompassing the 3' end of the *yocH* coding sequence, was isolated from one of the transformants.--